MULTIPLE INTERACTIONS AND COMPLEX VISCOSITY: THE IMPACT OF HIGH FREQUENCY RHEOLOGY FOR THE DEVELOPMENT OF HIGH CONCENTRATION PROTEIN FORMULATIONS

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Key Words: Viscosity, Protein-Protein Interaction, Formulation Development, High protein concentration

Clinical doses of therapeutic proteins range up to 2 mg/kg bodyweigth per patient and even higher. For patient convenience and competitiveness, subcutaneous (s.c.) applications are required. Therefore, liquid formulations for s.c. applications can reach concentrations of up to 200 mg/ml. One key parameter for the development of biotherapeutics as high concentrated liquid formulations (HCLF) is viscosity. Consequently, high solution viscosity is challenging due to e.g. impeded syringeablitiy and injectability that directly link to patient inconvenience, and high shear stress that potentially impair protein inherent stability. Following Jezek et al. 2011, we consider protein concentrations of >100 mg/ml as “highly concentrated”.

During early phases in development of biopharmaceutics only limited material is available. Therefore, prediction of the solution viscosity at higher concentrations (e.g. for HCLF conditions), if required, will be of great benefit. In this study, we applied different approaches comprehensively investigating parameters describing protein-protein interaction, protein hydration, protein conformation at different concentrations, and the volume fraction of the protein molecule in solution. At a molecular level, Protein-Protein Interaction (PPI) are a result of electrostatic-interaction, van-der-Waal (vdW)-forces and hydrophobic forces of bi- or multimodal interaction as well as protein-excipients interaction. At the macroscopic level, these parameters describe a crucial influence on the protein-stability and its rheological behavior in solution. However, during formulation development commonly evaluated PPI parameter such as the second virial coefficient (B_{22}), and/or the concentration- dependent diffusion coefficient (k_D). These parameters only describe interactions in dilute conditions, which poses limitations in predicting interactions at high protein concentrations. At dilute conditions, mostly electrostatic double layer repulsion and charge-shielding effects of buffer and excipients components dominate. In contrast, at high concentration, distances between individual molecules are narrowed, and thus attractive forces such as vdW interactions are predominantly present. Therefore, a direct correlation of PPI parameter obtained from dilute to crowded conditions is only a shaky compromise. The mechanisms and principles driving the formation of highly viscose systems are not fully understood, especially at the molecular level. As a consequence, the attempts to reduce viscosity are often left to chance. In a case study, we evaluated the behaviour of concentrated protein formulations under high-frequency shear excitation in the MHz range. As a result, we propose an explanation for interaction potentials between individual protein molecules linked to high solution viscosity by extending the complex colloid theory. Multiple attraction forces result in a complex viscous behavior, the formation of a transient micro-rheological network of multiple interacting protein molecules, and the formation of an elastic modulus. In order to lower the viscosity, such multiple interactions have to be disrupted and disordered by different excipients.

However, the interaction potential is correlated to the characteristics of each mAb molecule and can be altered by excipients in a defined way. By relating low concentration PPI measurements and wet-lab determined molecular characteristics (e.g. effective surface charge, dipole moment) it is possible to predicts the potency for describing a high viscosity for each mAb. Knowing the effects of pH and different buffer and excipients, a guided development for decreasing the viscosity by different excipients and formulation conditions is possible. High frequency rheology allows a rapid and early evaluation of the viscosity properties of early candidates and thus support subsequent formulation development.